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Strategies applied to modify structured and smooth surfaces: A step closer to reduce bacterial adhesion and biofilm formation

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ABSTRACT

Nearly a century has passed since the discovery of the first antibiotics. With each passing decade, more bacterial strains developed resistance towards existing antibiotics. Alternative methods to reduce contamination by bacteria and biofilms have arisen to reduce the pressure on existing or currently developed antibiotics. This review highlights promising approaches to prevent bacterial contamination of the surface. Special attention is paid to antibiotic-free antibacterial strategies that are not affected by bacterial resistance. The approaches have been divided into four categories: (i) anti-adhesive, (ii) contact active, and (iii) biocide attached/biocide release, which can be integrated with (iv) topographical modification. Anti-adhesive approaches can reduce the adhesion between bacteria and a solid surface to prevent bacteria from contacting and contaminating the surface. Contact active approaches provide antibacterial activity by attachment of antibacterial agents to the substratum. Biocide attached/biocide release integrates contact-release of toxic chemicals to bacteria attached to the surface. Lastly, topographical modification relies on approaches to produce small structural features capable of matching cellular components killing bacteria. Combining one or more antibacterial strategies can lead to a more robust approach to deal with dangerous pathogenic bacterial species. In this case, a way forward is by combining various coatings onto topographically modified surfaces, enabling multifunctionality to reduce adhesion and biofilm formation. A perspective on the current antibacterial surface challenge is provided.

1. Introduction

Since the discovery of penicillin by Alexander Fleming in 1928, humankind has been using antibiotics to treat infections [1]. Nearly a century has passed, and first-line antibiotics cannot cope with the adaptation mechanisms of bacteria, which ultimately develop antibiotic resistance [2]. The world needs to change the way of prescribing and overusing antibiotics [3]. Even if the new generation of antibiotics prevails, still providing antibacterial protection, antibiotic resistance will remain a significant human threat [3]. An urgent quest for alternative methods to reduce surface contamination by bacteria species has arisen to minimize antibiotic dependency [4]. In these alternative methods, bacterial contamination can be reduced by developing single or multi-level functionalization steps over surfaces to achieve exceptional antiseptic potential [5], preferably at the early stages of bacterial adhesion before the biofilm is formed [6].

In view of the variety of single or multi-level functionalization strategies, in the current paper, we categorize antibacterial surfaces as anti-adhesive, contact active, and biocide attached/biocide release. Anti-adhesive surfaces can reduce the adhesion between bacteria and a solid surface to remove bacteria before bacterial adherence and proliferation. Anti-adhesive surface strategies in this report include approaches using passive polymers, hydrogels, and poly-zwitterionic polymers. The active contact approach displays antibacterial activity by

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attachment of antibacterial agents to the substratum's surface. Contact active surface strategies include approaches on active action polymers, quaternary ammonium compounds, surface-attached antimicrobial peptides, and quorum sense inhibitors. Unlike the active contact approach, the biocide attached/biocide release approach integrates a toxic bacteriostatic or bactericidal substance. It can, therefore, be considered toxic by design. Attached/Released chemical or biological components, which are known to be prone to bacterial resistance, are not considered. The main focus of the presented paper is to provide a general overview of existing antibacterial strategies (e.g., coatings) applied to topographies. These functionalization approaches linked to topographical designs might be the stepping stone to reduce bacterial contamination, proliferation, and dangerous human infections [7–9]. In this respect, such functional topographies should reduce bacteria cell viability or cause cell death without promoting bacterial resistance. Despite the antibacterial mechanism imposed by topography, dead bacteria can always build up on the surface, inactivating bacterial killing properties of the surface [10]. Therefore, multipotent platforms to reduce cell adhesion and biofilm formation have yet to be found, particularly for long-term applications. Promising strategies that combine self-cleaning properties and bacteria-killing are an example of multipotent antibacterial platforms [11–15]. With such platforms, both bacterial killing and active bacterial detachment could be expected, maintaining the surface bacteria-free without the need for antibiotics and other hazardous chemicals to be released to the environment. Similar multipotent platforms that combine single or multi-level functionalization strategies are assessed. Strategies applied over topographies, particularly for long-term applications in the health care setting, are the main focus. Finally, a perspective on the current long-term antibacterial surface challenge is provided.

2. Biofilm formation

The long-term application of antibacterial surfaces is threatened by irreversible bacterial attachment, leading to biofilms. Biofilms have been identified as a possible source of infection. Verderosa et al. defined biofilms as complex three-dimensional communities of microorganisms adhering to a surface and encased in a protective extracellular polymeric substance (EPS). EPS is composed of protein (<1-2%), DNA (<1%), polysaccharides (1-2%), RNA (<1%), and water (up to 97%), being the main source for flow of nutrients inside a biofilm matrix [16]. Arunasri et al. mentioned the development from planktonic bacterial cells into sessile aggregates known as biofilm [11]. The proposed biofilm growth mechanism is divided into four biofilm formation stages, as shown in Fig. 1 [17,18].

In stage 1, the reversible attachment of bacterial cells to a surface occurs. During this stage, the free-floating planktonic cells identify a surface, where once landed, they can initiate the process of surface interaction and attachment. The attachment of planktonic cells to the surface is not permanent. Cell locomotion with the use of flagella or pili is granting preferential selection for surface attachment. In Stage 2, biofilm formation, irreversible attachment, and cell wall deformation over the substratum surface are typically observed. This process is mediated by the expression of quorum-sensing signaling molecules and by the formation of extracellular polymeric material. Stage 3 involves the formation of a mature biofilm with a 3D structure containing cells packed in clusters with channels between the clusters that allow transport of water and nutrients and waste removal. Unpredictable properties like resistance to external chemicals are an adaptative biological process of bacteria and are probably supported by the 3D network. Once the 3D-structured biofilm network is created, stage 4 takes place. Here, detachment and dispersion of cells from the biofilm and initiation of new biofilm formation occurs. Dispersed cells are morphologically more similar to planktonic cells than mature biofilm cells, which can initiate the biofilm development process again [19]. In other words, the spreading of infectious bacterial cells might commence.

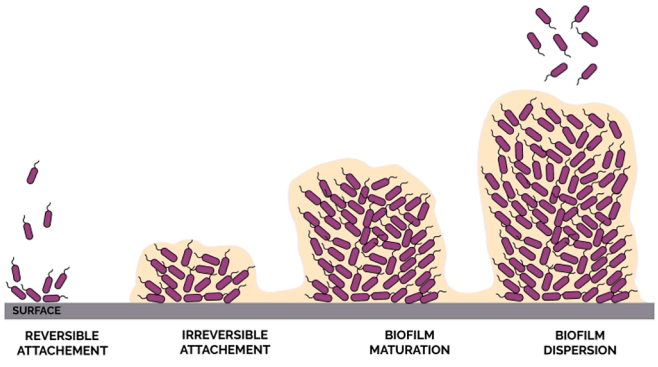

Fig. 1. Process of biofilm formation: reversible attachment, (i) irreversible attachment, (ii) 3D biofilm formation (iii), biofilm dispersion (iv) adapted from Maunders et al. [17,18].

In the following steps, we explore surface modification approaches employing anti-adhesive, contact active, and biocide attached/biocide release strategies. Surface modification strategies (mainly chemical) are then connected to functionalization parameters that can be end-compatible with topographical micro-/nano-structures fabricated using physical modification approaches presented in Table 1. In some cases, such topographies possess inherent antibacterial properties as well. In both cases, chemical and physical approaches are shown in Fig. 2, where the common goal is to reduce bacterial attachment or prevent biofilm formation. The implemented level of functional strategies is described below.

2.1. Surface modification strategies

Biofilm formation is the survival strategy of microorganisms wherein microbial cells adapt to their environment and to a multicellular lifestyle in which bacterial cells are self-immobilized in a matrix of extracellular polymeric substance (EPS) [19,20]. The bacteria inside this matrix are shielded against antibacterial compounds and are up to 1000 times less susceptible to antibiotics [21]. Thus, the prevention of the early stages of biofilm should be the primary focus (i.e., stages 1 & 2 during biofilm formation, shown in Fig. 1).

A key issue identified for the recently developed chemical strategies is that, as time goes by, the substratum's degradation occurs as a result of chemical agent depletion or loss of structural surface integrity. Consequently, an opportunity for bacteria to adhere to and proliferate is created. Biofilm formation can occur on virtually any surface. Most of the strategies shown in Table 1 render to surface contamination. In the ideal case, most of the surfaces should last long. Nevertheless, very few of them have been identified to retain their antibacterial properties over extended periods [22].

As shown in Fig. 2, an overview of the division of antibacterial strategies is given. Here, a division is made between physical and chemical strategies. Chemical strategies, such as anti-adhesive (AA), contact active (CA), and biocide attach/release (BAR), include treatment-induced on the surface, i.e., polymerization and surface functionalization. The second group includes physical treatment involving modification of the surface topography (TM), changing surface properties, and creating a superhydrophobic or hydrophobic interface for self-cleaning or slippery interfaces. Each of the identified antimicrobial strategies contains several subgroups listed in Table 1. This table describes AA, CA, BAR, and TM approaches with a general remark when integrated to topography, such as functionalization compatibility.

The main themes identified can be formulated as follow:

Table 1
Multifunctional chemical and physical strategies required to reduce bacterial attachment and biofilm formation.

Strategy	Subcategory	Approach	Remark	Compatibility with topographies	References
Chemical Strategies	Anti-adhesive	Passive polymers, Hydrogels, Poly-zwitterionic polymers.	Mechanisms based on steric exclusion repulsion, electrostatic repulsion, and low surface energy.	Yes, as a coating.	[23–26]
	Contact active	Quaternary ammonium compounds, Surface attached antimicrobial peptides, Quorum sensing inhibition.	Contact active agents induce bacterial cell death or reduce the metabolic activity of bacteria, minimizing their pathogenic effect in their biological environment.	Yes, as a coating.	[27–29]
	Biocide release	Nanoparticle Metals;	Release of an active agent inducing bacterial cell death or interferes with bacterial cellular interactions.	Yes, as a composite.	[30]
Physical Strategies	Topographic modification	Superhydrophobic moieties, Hydrophobic moieties.	Inhibition of biofilm formation through superhydrophobic hydrophobic interaction.	Yes, but complicated	[31,32]

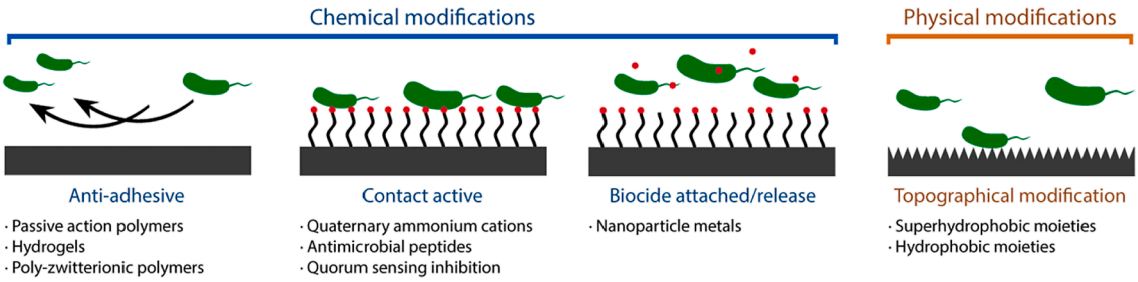

Fig. 2. Surface modification strategies for antibacterial application.

(i) AA: involves mainly passive action polymers, hydrogels, zwitterions motives, the action of which is based on steric exclusion repulsion, electrostatic repulsion, and low-surface energy.
(ii) CA: relates mainly quaternary ammonium cations, antimicrobial proteins, and peptides, and quorum sensing inhibition, the action of which is based on the covalent attachment of agents which can induce bacterial cell death or can reduce bacterial metabolic activity.
(iii) BAR: involves mainly nanoparticles, which is based on the loading and release of an activity that can kill bacteria or reduce its metabolic activity.
(iv) TM: addresses hydrophobic or superhydrophobic moieties based on the inhibition of bacteria due to hydrophobic interaction.

3. Chemical strategies

With the use of chemistry, surfaces can be modified to provide a specific function. In this section, various methods and development regarding the fabrication of antibacterial biocide-free surfaces are described. Three of the four main strategies are approaches connected with chemical modification techniques.

3.1. Anti-adhesive principle

The principle of an anti-adhesive surface is an effective strategy to reduce biofilm formation. Anti-adhesive surfaces can reduce the adhesion force between the bacterium and the substratum, preventing biofilm formation. The mechanism of repulsion of bacteria, as shown in Table 2, is based on exclusion steric repulsion, where repulsion occurs when polymers attached to coating surfaces provide physical barriers to proteins from the bacteria cell wall [23]. Another mechanism is electrostatic repulsion, where repulsion occurs due to charges on coatings preventing the attachment of microbes [24]. Lastly, the mechanism of low surface energy where reduction of external microbial adhesion occurs due to low energy surfaces [25,26]. With these three main mechanisms in mind, strategies on anti-adhesive approaches like passive action polymers, hydrogels, poly-zwitterionic polymers are next introduced. Each of the strategies relies on one or a combination of low energy and repulsion mechanism.

Table 2
Physicochemical anti-adhesive mechanisms.

Mechanism	Based on
Exclusion steric repulsion	Repulsion by physical barriers to proteins, cells, and microbes.
Electrostatic repulsion	Repulsion by electrostatic charges of molecules/coatings.
Low surface energy	Reduction of external microbial adhesion due to low-energy surfaces.

3.1.1. Passive action polymers

Today, various types and subclasses of bacterial adhesins are described in the literature as active polymers [33]. Bacterial adhesion depends primarily on intramembranous structural proteins found on hair-like appendages such as pili, fimbria, and nanofibers, which provide a scaffold upon which several extracellular adhesins may be attached [33,10]. In many cases, these adhesins are minor subunit proteins at the tip of fimbria [33,34]. It is possible to reduce bacterial protein adsorption on its surface with passive polymers, thereby preventing bacterial adhesion. Passive polymers generally include self-healing, slippery liquid-infused porous surface (SLIPS) [35,36], uncharged polymers [23], charged polyampholytes, and zwitterionic polymers[37].

Among the passive polymers, Poly(ethylene glycol) (PEG) is the most commonly used. The use of surface-immobilized PEG and its derivatives for antifouling activity are assessed. Chirife et al. hypothesized the antibacterial action of PEG-400, attributing to two effects, lowering the water activity and the specific action of PEG-400 molecules on bacterial cells [38]. Afterward, Jeon et al. proposed that repulsive forces hinder bacterial proteins, which can most probably be electrostatic forces [39], resulting from highly mobile PEG chain compression on functionalized surfaces. Furthermore, this theory proposes that the compression of polymer chains would need the thermodynamically unfavorable removal of water molecules from the hydrated polymer. This would result in a formation of a tightly bound water layer interacting with the grafted polymer, acting as a physical barrier to the adsorption of protein and bacteria [40]. Benčina et al. reported that a precise antibacterial repellence mechanism of surface-immobilized PEG is still not fully

elucidated. However, various studies report numerous properties of the grafted polymer, including grafting density, chain length/thickness of the polymer layer, conformation, and wettability which play an essential role in resisting protein adhesion [25].

Ali-Ani et al. demonstrated a reduction of adhesion by tuning the grafting density of PEG chains. In this study, (3-Aminopropyl) triethoxysilane-grafted silicon wafers were used to attach PEG chains of different densities covalently. Here, a systematic method of grafting density is demonstrated, linking the polymer density to the solution's salt concentration (K_2SO_4), fabricating the highest possible density at 0,6M/60 °C. These PEG-modified surfaces showed less bacterial adherence than Si and APTES surfaces concluding that this reduction is attributed to the hydrophilicity of covalently grafted PEG. The results are in agreement with experiments where APTES is used to functionalize Si or SiO_2. The results demonstrate that regardless of the hydrophilic character of the functionalized with APTES, bacteria still adherence and proliferate [41]. In the case of eukaryotic cells, such as mesenchymal stem cells [42] and MG63 human osteosarcoma cell attachment was increased at low and medium PEG-polymer densities [42]. Jiang Wu et al. investigated the effect of molecular weight of PEG and mass ratios of PEG: proteins on the interactions between PEG and proteins in aqueous solution. In this work, Jiang Wu et al. concluded that long-chain PEG could interact with proteins in an aqueous solution. PEG–protein interactions induce conformational changes to relatively open structures with increased hydrophobic cavities, provide multiple binding sites of proteins to PEG, and facilitate a PEG–protein complexes formation. The effect is often neglected for long-chain PEG. However, short-chain PEG behaved differently and indicated little tendency to protein interaction due to its inability to form multiple binding sites [43]. Zhang concludes that though the antifouling property of PEG displays better functionality than most other hydrophilic polymers, the stability of PEG is impaired due to oxidation of its intrinsic ether linkage.

PEG has shown reliability in various applications in the medical field owing to its antifouling properties, biocompatibility, and excellent safety [44–46], and is used, e.g., for coating of implants [47]. Although PEG is one of the most commonly used passive polymers, known for its properties regarding PEG-protein interaction in aqueous solution, its mechanism of action and antibacterial properties are not fully understood and should be investigated more thoroughly. An interesting aspect to explore is the dependence of PEG stability on chain length, particularly when imparting antifouling functionality. Eventually, PEG can find application in the health care setting used, e.g., indoor handles, linen, and clothing [48]. PEGylation (attachment of PEG to surfaces) has been a “golden standard” to resist nonspecific protein adsorption [49]. However, it has been reported that the hydrophobic character tested in-vivo, particularly with highly immunogenic protein conjugated to PEG, can generate PEG-specific antibodies. The generation of these antibodies in term causes the complete elimination of subsequent doses of PEGylated agents [49–51]. Although recent studies have identified the chemical origin of PEG, the exact biological mechanism of PEG immunogenicity is not completely clear [51]. As a result, PEG might not be safe for all applications in health. PEG’s immunogenicity might limit the scope, and more research must be in place to overcome its limitations [52].

3.1.2. Hydrogels

Hydrogels are three-dimensional (3D) polymer networks cross-linked by physical or covalent bonds [53]. These gels are a class of highly hydrated material finding use in a diverse medical application for its general biocompatibility properties. As a result, hydrogels make a convenient platform to develop selectively active antimicrobial materials [54]. Hydrogels have, compared to other types of biomaterials, distinct properties such as high water content, controllable swelling behavior, and biocompatibility [55]. Salome Veiga et al. described two types of antimicrobial hydrogels: hydrogels either encapsulating or covalent immobilizing antimicrobial agents or hydrogels with an inherently antimicrobial activity where the matrix itself displays antibacterial activity [54]. The main advantage of using hydrogels is the control of specific properties such as morphology by controlling the number of crosslinkers and monomers in the hydrogel network [56], thereby influencing the ability of dispersion of loaded agents and its antibacterial activity. Wang et al. described that the active antifouling mechanism strongly depends on the environment media due to the complicated interactions between the fouling agent (foulant), antifouling materials, and the solvent media. However, whether a surface will be antifouling toward the adsorption of a foulant is understood by standard thermodynamic considerations [36]. In this case, the free energy of the adsorption process contains an enthalpic component describing the strength of interactions between the foulant, solvent, and surface [36]. Hydrogels with inherently antibacterial properties include hydrogels made of chitosan, peptides, and polymers [57,58]. In this work, peptide-based hydrogels will not be discussed further.

3.1.2.1. Chitosan-based hydrogels. Chitosan (CS) is a linear polysaccharide. CS is able to polymerase by cross-linkage in the presence of anions and polyanions [59]. A common method to make CS is by deacetylation of Chitin (CT) [60]. CS-based hydrogels have shown properties such as self-healing, antibacterial activity, biocompatibility, and biodegradability [22,61,62]. Ravishankar et al. divided chitosan-based hydrogels into three generations: the first generation comprises chemically and metal coordinated cross-linked hydrogels. These cross-linked hydrogels result in fair-to-good mechanical properties. However, the majority of the first generation is toxic. The second generation comprises physically cross-linked hydrogels utilizing physical forces, such as electrostatic interactions, H-bonding, and hydrophobic interaction. In contrast to the first generation, the second generation hydrogels are non-toxic, demonstrating low-to-moderate mechanical properties. Lastly, the third generation comprises extensive physical cross-links across the chitosan chains. These hydrogels demonstrate very high stiffness and exceptional environmental stability, overcoming the drawbacks of the second generation. Common examples of third-generation hydrogels include polyanions having a large molecular weight and a net charge ratio. This generation can be shaped as nanoparticles end-having a negatively charged surface. However, it should be noted that the third generation needs further research as the antibacterial mechanism of action is not fully elucidated [63].

CS can inhibit bacterial growth through positively charged amino groups interacting with the negatively charged cell outer membrane [64]. The ability to attach CS hydrogels to surfaces might represent an exciting option to achieve antibacterial properties using surface functionalization approaches. For example, methods to covalent attach hydrogels to a surface can vary. He et al. reported a bilayer hydrogel coating that can switch from a cell-adhesion mode of action to an antibacterial mode of action. To create the coating, firstly, a CS hydrogel thin film is covalently attached to a thiol-modified substrate via the thiol-one click reaction of an ene-functionalized copolymer of poly (sulfobetaine methacrylate-acrylate acid-2-hydroxyethyl methacrylate) (P(SBMA-AA-HEMA)). After that, heparin-mimicking polymer chains were grafted onto the hydrogel thin film layer via surface-initiated atom transfer radical polymerization [65]. In a later study, He et al. demonstrated a robust method to covalently attach multifunctional hydrogel thin layers onto substrates showing reliable stability. Thin hydrogel layers were formed and covalently immobilized onto substrate surfaces, as shown in Fig. 3, by two steps: double bonds introduced onto the substrate providing the surface with anchoring points for hydrogel layers, and the formation and simultaneously attachment of hydrogel layers onto the substrate surface by cross-linking copolymerization for the double bonds and functional monomers. Here, hydroxyl groups on polyethersulfone (PES) substrates were prepared by cross-linking polymerization of HEMA followed by a phase inversion technique. Via the reaction of acryloyl chloride and the hydroxyl groups, double bonds

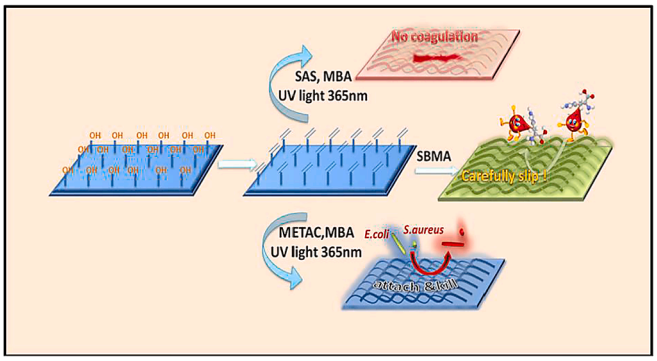

Fig. 3. A thin hydrogel layer with anticoagulant, antifouling, and antibacterial functions. The synthesis procedure of surface-attached hydrogel thin layers is highlighted [66].

were introduced, which provided anchor points for the hydrogel layers. Then, various precursor solutions were added to coat the double bond surface-covered PES substrates, respectively, and cross-linking copolymerization was executed at 365 nm to attach the hydrogels covalently. Calculations of the residual masses of the samples were made to evaluate the stability of the layers. The results indicated high stability as residual masses remained unchanged after seven cycles of treatment. Interestingly, He et al. noted that for metal and inorganic substrates, anchoring sites can be provided by dopamine chemical coating or treatment with fresh piranha. Thereby showing the versatility of this technique [66]. In a different research, Bidhari et al. covalently bond antifouling thin layer hydrogels on 3-ethoxybenzophenonesilane-modified inorganic or bare organic substrates by irradiation under UV light. Due to irradiation, photo-active benzophenone molecules enabled the generation of the polymer network and the attachment of the network onto the substrates.

In general, the covalent attachment of hydrogels to surfaces has been proven successful by various strategies. The ability to covalently attach thin hydrogels may provide surfaces an antifouling or antibacterial functionality depending on the hydrogel used. Moreover, the addition of thin layer hydrogels may increase the antibacterial functionality of inherent antifouling or antibacterial surfaces. Ultimately, properties, e.g., biocompatibility and tunable biodegradability, can be provided to these surfaces showing their versatility for the medical setting. However, further optimization of these techniques is needed [67], especially when applied to topographical surfaces.

3.1.2.2. Nanoparticle incorporated hydrogels. For antibacterial surface applications, nanoparticle incorporated hydrogels are an option of choice, particularly when in contact with the body (i.e., wound dressings). These materials could also include antibiotics. However, in this paper, antibiotics will not be described as it is not the focus of the current literature review. Bodnenberger et al. describe the use of NP applications in hydrogels. In this case, the hydrogel is loaded with NPs enabling to increase antibacterial efficacy [68]. Among metal NPs, silver NPs (AgNPs) have attracted much research due to their antimicrobial properties [69,70]. AgNPs in solution, at the surface or composite, can lead to various antibacterial mechanisms, e.g., the generation of reactive oxygen species (ROS), which are harmful to bacteria [71]. ROS, such as superoxide, hydrogen peroxide, and hydroxyl radical, can cause several types of intracellular damage in bacterial cells [72]. When in contact with mammalian cells, some formulations of AgNPs can be cytotoxic, limiting their application in humans [73,74]. However, exceptions exist in the literature for AgNPs alone [75–78] and AgNPs-hydrogels nanocomposites with non-cytotoxic effects [79].

Niu et al. reported the preparation of supramolecular hydrogels hybridized with AgNPs by *in-situ* reduction of $AgNO_3$ stabilized by PPEGMA-ran-PAA followed by complexing with α-cyclodextrins (α-CDs), as shown in Fig. 4. These hydrogels were physically cross-

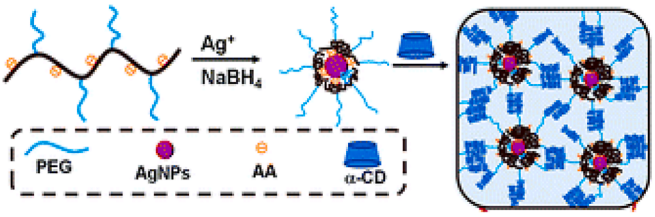

Fig. 4. Schematic procedure for the preparation of AgNPs hybrid supramolecular hydrogels [80].

linked by both pseudopolyrotaxane crystallization and AgNPs, which showed temperature responsiveness properties. Due to hybridization, the hydrogels showed excellent antibacterial properties against *S. aureus* and *E. coli* bacteria showing potential applications as injectable antibacterial materials [80]. Garcia-Astrain et al. developed a bionanocomposite hydrogel based on gelatin and chondroitin sulfate with covalently attached AgNPs. In this study, Garcia-Astrain reported using maleimide-coated AgNPs as cross-linkers to prepare a bionanocomposite gelatin-based hydrogel via Diels–Alder cycloaddition to furan-modified gelatin [81]. Despite their biocompatibility, toxicity was not entirely reduced. The results indicate that synthetic methods can change NPs toxicity and should be tunable to maintain antibacterial activity and reduce cytotoxicity towards human cells [75]. The advantages of incorporating NPs into hydrogel have great potential to increase material functionality in the clinical setting.

3.1.3. Poly-zwitterionic polymers

Zwitterionic polymers refer to a family of materials with equal cations and anions along their polymer chains [82]. These polymers contain positive- and negative charged groups incorporated into their structure, making them highly hydrophilic antifouling compounds [83]. Classified by anions, the zwitterionic groups can be classified into sulfobetaine (SB), carboxy betaine (CB), phosphorylcholine (PC). Zheng et al. report that due to the favorable antifouling capacities provided by the chemical groups, zwitterionic materials could have applications in biomedical devices, implants, drug delivery, separation membranes, and marine coating. However, the application is still made at a laboratory scale, and applications at the industrial scale still see many challenges [82].

SB-based polymers are commercially available monomers and with more applications found in the literature. CB-based polymers have shown excellent antifouling properties [84] and biocompatibility [85]. PC-based polymers show excellent biocompatibility [37,82] compared to SB- and CB-based polymers. Zwitterionic hydrogels exhibit unique behaviors and properties, including “anti-polyelectrolyte” behavior, unusual pH sensitivity, and temperature sensitivity [82]. Research has shown that polyzwitterion's antifouling action cannot be correlated to the type of zwitterion used but due to their exact chemical structure [86]. Shen et al. demonstrated that soft and wet drag-reducing zwitterionic hydrogel coatings have weak swelling in saline solution and are effective against *E. coli* and *S. aureus*. The antibacterial activity and weak swelling were obtained by the combination of using the anti-polyelectrolyte effect of poly-*N*-(3-sulfopropyl)-*N*-(methacryloxyethyl)-*N*,*N*-dimethylammonium betaine (PSBMA) and the typical polyelectrolyte effect of polyacrylic acid (PAA) [87]. Although PSBMA exhibited antifouling properties, PSBMA has an anti-polyelectrolyte effect, which mechanism is shown in Fig. 5 [88], causing swelling and shrinkage behavior when transferred from water to a saline solution. However, this effect was suppressed by using PAA, improving the mechanical properties of the hydrogel [87]. Huang et al. increased the durability of zwitterionic polymers by blending networked zwitterionic microgels with the PES polymer matrix. For both *E.coli* and *S.aureus* a reduction in bacterial attachment has been observed. Furthermore, the antibacterial property was maintained after challenges with harsh

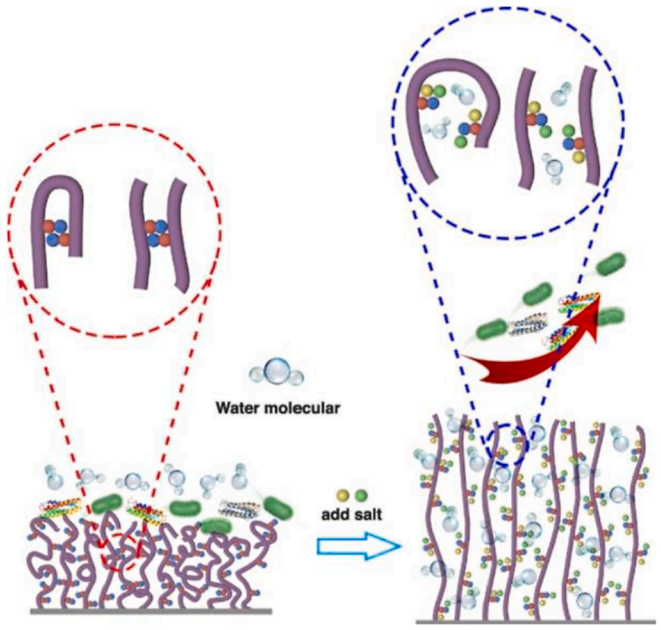

Fig. 5. Mechanism of anti-polyelectrolyte effect of zwitterionic polymer brushes [88].

chemical environments [89].

An attractive system is the combination of polyzwitterions and PEG, which has shown antifouling property [90–93]. However, the exact mechanism has not been elucidated. A deeper understanding of non-fouling performance is needed. Leng et al. compared the surface hydration of antifouling zwitterionic and PEG materials in contact with proteins. Results suggested that surface hydration for PEG-coated surfaces and NPs, are relatively strong and resist protein adsorption. This is due to surface hydration that is disrupted to a certain degree. Free-floating in solution PEG showed bondage with proteins which reduces hydration. SB-coated surfaces, or free-floating SB, showed strong hydration in contact with proteins [93]. As evident from the research studies, zwitterionic polymers show antibacterial and antifouling properties achieved by their strong hydration in contact with proteins. The diversity of zwitterionic polymers used is still limited. More research is needed to broaden the existing structural diversity of polyzwitterions for antifouling optimization. The next generation of zwitterionic polymers should take into account mechanical stability at the surface over a prolonged time without losing antifouling properties. When applied in the clinical setting, specifically in contact with the human body, biocompatibility should be assessed.

3.2. Contact active principle

Unlike the anti-adhesive principle, where antifouling mechanisms reduce bacterial adhesion, the active contact approach focuses on immobilizing antibacterial agents onto the surface through covalent bonds that kill bacteria on contact. Strategies based on the principle of active contact include the use of active action polymers, quaternary ammonium cations, and antimicrobial proteins & peptides.

3.2.1. Active action polymers

In contrast to passive polymers, which are based on their antifouling ability, active polymers are based on killing bacteria that adhere to the polymer surface [94]. Typically, these polymers are functionalized with an antibacterial agent that kills or reduces the metabolic activity of bacteria, minimizing their pathogenic effect in their biological environment. The exact mechanisms of these functionalized polymers, however, depend on the active agent. Nowadays, the most widely used active antimicrobial polymer is functionalized with positively charged quaternary ammonium [94].

3.2.1.1. Quaternary ammonium compounds. Quaternary ammonium compounds (QACs) are positively charged polyatomic ions [95]. They are among the most commonly used disinfectants in the food industry for long-term stability and effectiveness against bacterial biofilms [27]. QACs consist of 4 alkyl groups attached to a central cationic nitrogen atom [95]. By nature, QACs are perpetually charged, regardless of the pH solution [95]. Various known factors contribute to the antibacterial effect of QACs. For instance, the antibacterial efficacy depends on their chain length and can be grouped into long- and short-chained [96]. In this review, the definition of long-chained QACs by Li et al. [97] and Kaur et al. [96] is where their alkyl substitution reaches longer than six. The optimum chain length for antibacterial activity of QACs for gram-positive bacteria is 12-14 carbons and a length of 14-16 carbons for gram-negative bacteria [98,99]. However, the exact mode of action of immobilized QACs has yet to be explained. It is known that a threshold of molecular charge density of immobilized QACs is required to induce cell death [99,100]. The charge density threshold (quaternary amine units/cm^2) for contact killing in high-division conditions (i.e., log-phase) is 10^{12} and 10^{13} N^+/cm^2 for *E. coli* and *S. epidermis* [101]. As for low-division conditions (i.e. stationary pahse) it is 10^{14} N^+/cm^2 for both *E. coli* and *S. epidermis* [101].

To covalently integrate QACs onto biomaterial surfaces, various methods have been developed, which include the sol-gel process via covalent hydrolyzable ester linkage, atom transfer radical polymerization, plasma polymerization, and layer-by-layer deposition [102–107]. The approach is effective to impart permanent active contact antimicrobial activity on surfaces [105,108–114]. In 2013, Asri et al. demonstrated a coating of covalently tether QACs in a hyperbranched configuration onto silicon (Si) surfaces. As shown in Fig. 6, these surfaces were prepared by covalently attaching hyperbranched polyurea (HB) coatings to glass slides. Polyethyleneimine (PEI) was added to couple covalently with the polyurea branches. By consecutive alkylation with 1-bromohexane and iodomethane the coatings were converted into hydrophobic polycationic species creating. As a result, a-Si-HB-PEI^+ coating. Here, two concentrations of PEI was used, 10 wt% and 20 wt% [115]. Evaluation by confocal microscopy showed that coatings made with a 10 and 20 wt% concentration of PEI showed a charge density of 6 $\times$ 10^{15} and 4 $\times$ 10^{15} N^+ cm^{-2}, respectively. The 20 wt% Si-HB-PEI^+ coating displayed >99%, >99,9% and 99,99% contact-killing at bacterial challenges of 16, 160 and 1600 CFU/cm of *S.epidermidis*.

In 2019, Dong et al. demonstrated similar research of immobilized robust hyperbranched antibacterial coatings on poly(dimethylsiloxane) (PDMS) [116]. These coatings showed exceptional antibacterial activity against various Gram-positive and Gram-negative bacteria. Interestingly, by utilizing EDTA as a permeabilizer, the antibacterial activity was strongly enhanced by weakening the molecular interactions of the lipopolysaccharide constituent of the outer cell membrane [117], displaying similar effectiveness for both Gram-positive and Gram-negative bacteria [116]. Unlike tethering QACs to hyperbranched polymers, Villanueva et al. concluded that medical grade poly(vinyl chloride) (PVC) is able to transform into antibacterial plastics with high antibacterial activity against both gram-positive and gram-negative microorganisms. Here, a coating comprising of QACs and aliphatic moieties was formed on PVC. Mercaptopropyltrimethoxysilane and aminopropyltriethoxysilane were grafted onto PVC. Then betaine and dodecyl succinic anhydride (DDSA) were bonded to free amino groups. Surprisingly, surfaces treated with betaine and DDSA showed low bacterial attachment. The mode of action of the coated PVC is concluded to be related to the interaction between cationic and aliphatic moieties and microbial cells [118].

Although QACs are commonly used, there remains a lack of knowledge on the toxicity of surface-immobilized QACs, which should be

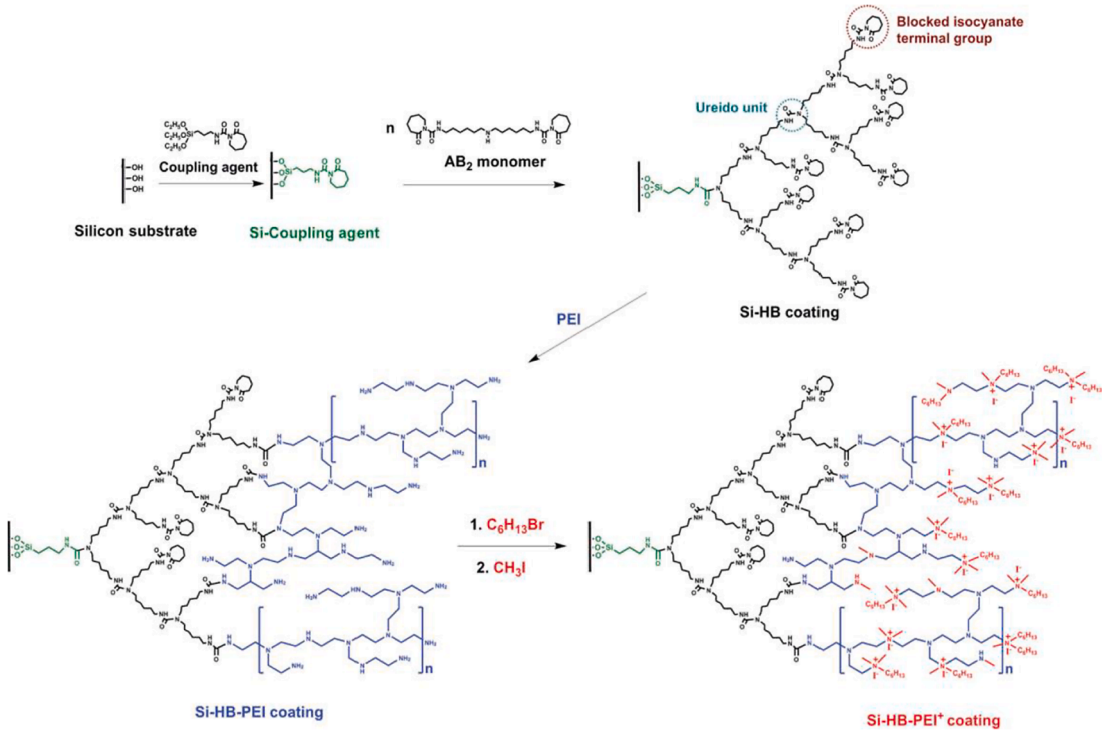

Fig. 6. Schematics of the preparation of covalently attached hyperbranched polyurea coatings, tethering of polyethyleneimine and two-step alkylation of PEI that was covalently coupled onto hyperbranched polyurea coatings [115].

crucial to research for creating and designing intelligent materials. QACs, though are simple in structure but are complex as various variables attribute to their antibacterial effect. However, the charge density of immobilized QACs, in particular, has proven to be crucial. As mentioned earlier, to induce cell death, a threshold charge density has to be met. Therefore, investigation for optimizing attachment density should be focused on. Surface immobilized QACs show high potential as an antibacterial surface strategy. However, the use of surface-immobilized QACs is still in the development stage. Therefore, the toxicity and safety application in medical settings remains uncertain and should focus on future research.

3.2.2. *Surface attached antimicrobial peptides*

Antimicrobial peptides (AMP) are oligopeptides composed of cationic and hydrophobic amino acids [28,119]. AMPs play a crucial role as potent antibiotics in innate immunity. They are categorized by their secondary structure in four groups, which include β-sheet, α-helix, extended, and loop [119]. The use of AMPs has emerged as a promising strategy in the combat against biofilm-related infections because they show high potency against biofilms. However, AMPs are limited in the clinical setting by a couple of factors, e.g., bio-degradation and lack of knowledge on the multiple mechanisms of action [28]. In recent studies, many mechanisms of action of AMPs have been described [120]. Although, it has yet to be determined whether the multiple mechanisms of AMPs are independent of one another [120]. It is hypothesized that surface attachment of AMPs can be target specific to the cell membrane [28]. However, unlike other antimicrobial agents, AMPs only exhibit antibacterial activity if orientated adequately on the surface [121–123].

Dutta et al. immobilized the antimicrobial peptides, LL-37, melamine, lactoferricin, and Mel-4 onto poly-hydroxyethyl methacrylate (pHEMA) surfaces to achieve an antibacterial effect. These findings indicated that a threshold concentration of immobilized AMPs on pHEMA is crucial to achieving antimicrobial activity. Furthermore, outcomes showed that the inhibition is highly sensitive to the attachment technique used. Interestingly, Dutta et al. noted that the contact area between the AMP and the bacteria affects the efficacy of the AMP functionalized surface [124].

Yasir et al. elucidated the mechanism of action of surface-immobilized antimicrobial peptides Melamine and Mel-4 against *P. aeruginosa*. Melimine and Mel-4 are chimeric cationic peptides inhibiting a broad- spectrum of antimicrobial activity. The two peptides are highly biocompatible and have been tested in human clinical trials. Yasir et al. concluded that both immobilized melamine and Mel-4 show similar mechanisms of action immobilized as in their solution-phase: the ability to bind bacterial lipopolysaccharides, cause membrane disruption, and facilitate the release of ATP and subsequently DNA/RNA from cells [125].

He et al. developed an immobilization method of AMPs with stable activity by combining initiated atom transfer radical polymerization on silicon surfaces (SI-ATRP) and click chemistry [121]. Generally, attachment of AMPs to a surface has limitations as stability is impaired due to degradation of the peptide by enzymes and antibacterial activity could be impaired if the AMPs are not correctly orientated due to surface energy [122,123,126]. By using a spacer molecule, as shown in Fig. 7,

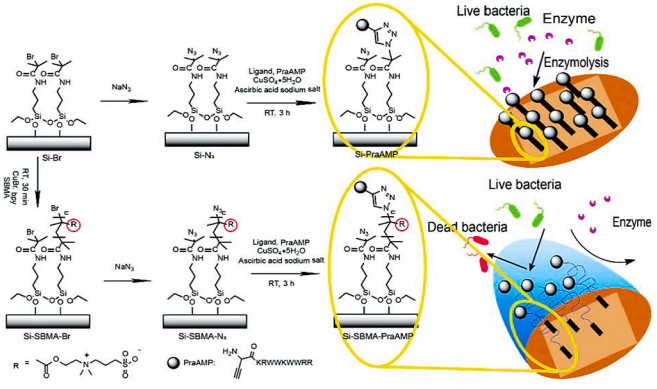

Fig. 7. Schematic diagram for the chemical immobilization of PraAMP to Si surface [121].

poly[2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammoniumhydroxide (polySBMA), enzymolysis stability of the surface was increased. Furthermore, by modifying the antimicrobial peptide HHC36 with L-propargylglycine (PraAMP) and attaching the PraAMP to the spacer molecule, salt-tolerant properties were enhanced. The surface exhibited antimicrobial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* and exhibited negligible cytotoxicity to mouse bone mesenchymal stem cells.

Novel techniques to combat biofilm formation have risen. Among the most promising antibacterial strategies, surface-attached AMPs are strategies with the most potential, as they have shown high potency against biofilm formation and good biocompatibility. Moreover, AMPs have shown high sensitivity to techniques used for surface attachment. In addition, the efficacy of AMPs has been revealed to be affected by contact area and orientation. Therefore, to gain more knowledge, for improvement and optimize efficacy, the diversity of AMPs should be tested on various attachment techniques and correlated AMPs attachment to the specific action mechanism. To date, AMPs in the clinical and medical application will stay limited unless the mode of action against specific bacterial strains is better understood. Yasir et al. has shown great steps in elucidating the mechanism of action of surface-immobilized Melamine and Mel-4 where results conclude that the immobilized form of both AMPs exhibits a similar mode of action to its soluble form. Nevertheless, the use of surface-attached AMPs may set a new standard in long-term antibacterial and even broader antimicrobial functionality for its potency and its intrinsic ability to combat biofilm.

3.2.3. *Quorum sensing inhibition*

Quorum sensing (QS) is a bacterial cell-to-cell communication process that relies on the production, sensing, and response to extracellular signaling molecules that allow bacterial communities to share information about their changing environment [127–129]. QS alters specific gene' expression in a population [129,130]. For example, QS in gram-negative bacteria makes use of the release of chemical signal molecules called autoinducers. Gram-negative bacteria use acyl-homoserine lactone (AHL) as an autoinducer [128,131]. When the population density of a bacterium reaches a specific "quorum," the corresponding level of AHL concentration can induce activation of transcriptional activators, which in turn activate transcription of genes and modify physiological functions [128,132].

3.2.3.1. Surface immobilized quorum sense inhibitors. Surface immobilized QS-inhibitors (QSIs) rely on inhibition of QS, resulting in blockage of bacterial communication rather than inducing bacterial death. Ho et al. demonstrated the reduction of bacterial surface adhesion up to 97% for both *P. aeruginosa* and *S. aureus* by covalent attachment of the QSI, dihydropyrrolones (DHPs). DHPs were immobilized on glass surfaces via copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition click reaction. This click-DHP coating displayed an exceptional reduction in bacterial adhesion and biofilm formation in vitro over 48 hours[29]. Taunk et al. compared the immobilization of various DHPs and furanone (FU) compounds on azide-functionalized glass surfaces. The results suggest that non-specific attachment of FUs and DHPs to highly reactive azide-groups does not impair the antibacterial activity of the compounds, indicating that the compounds retain their activity even after attachment[133]. In later work, Taunk et al. demonstrated the importance of the structural orientation of DHPs for *S. aureus* activity. Results displayed better antibacterial activity with surface attachment to the N-position opening the phenyl group (Fig. 8) rather than the attachment of C-4 position of the phenyl of DHP. However, results showed no difference in activity for *P. aeruginosa* [134].

In general, the immobilization of QSI's on surfaces has shown to be a novel strategy with a high potential for antibacterial application. The precise mechanisms of action of surface-immobilized QSI's have yet to be elucidated. Ho et al. has demonstrated the utilization of CuAAC click chemistry for specific covalent surface attachment of the QSI DHP. The structural orientation of DHP can influence antibacterial activity. Therefore, it is imperative to show the importance of the orientation and location where QSIs attachment occurs. However, before a clinical application, research is crucial as surface-immobilized QSIs are a novel strategy that has yet to be fully understood. Nevertheless, the ability to immobilize QSIs on surfaces represents a potential approach as an antibacterial coating for effective biofilm formation prevention, reducing the risk for the development of bacterial resistance.

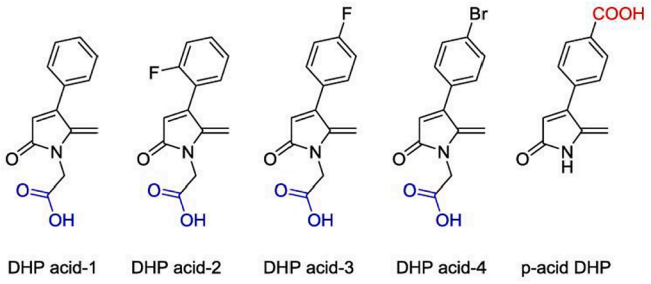

Fig. 8. Chemical structures of synthetic DHP derivatives [134].

3.3. *Biocide attached/release principle*

The biocide attachment and release approach integrates the release of a toxic substance to bacteria upon attachment [135]. There are multiple strategies where biocides can be attached and released to eradicate bacteria from the surface [130–135]. Some of them can be considered toxic by design, as in metals and other complex organic substances. Only a few of these approaches are not toxic [136], such as antifouling-based principles for marine applications. However, in the clinical setting, selective pathogenic bacteria-killing remains an issue. Efforts have started to appear for fungi using metal nanoparticles [70]. Similar strategies should be applied for the design of multifunctional surfaces selective to pathogenic bacteria. Besides the uniqueness of the proposed approaches, we will focus on metal nanoparticles attached/released from the surface and the principles of these processes in this section.

3.3.1. *Nanoparticle metals*

In this section, surface immobilization with metal nanoparticles is discussed [137]. Depending on the NP type, the antibacterial mechanism might vary [30]. A broadly accepted mechanism is the generation of reactive species (ROS) that can end-compromising the cell membrane integrity, as depicted in Fig. 9. Another critical parameter to evaluate is the antibacterial activity of metal ions in solution from metal nanoparticles which is generally non-specific, displaying a broad spectrum of activity [138], e.g., AgNPs.

To provide antibacterial functionality to a surface, metal NPs can be either covalently immobilized or coated onto surfaces. Moreover, NPs can be loaded into hydrogels and then be coated onto a surface to an antibacterial mode of action. Materials frequently used are silver, copper, gold, iron, and zinc in the form of NPs. In Fig. 9, hypothesized mechanisms of AgNPs and silver ions are displayed [139]. The generally accepted phenomenon is based on NPs causing damage to bacterial cell membranes or cause detrimental alterations to organelles [53]. Yeray et al. developed a versatile method of synthesis in situ of silver NPs with a well-defined size using maleimide as a single crosslinker and polyvinyl alcohol [140]. Yeray et al. concluded that the cross-linking degree of hydrogel networks regulated and stabilized the NP size in their work. In this study, the encapsulated AgNPs have demonstrated antibacterial activity against *S. aureus* owing to the release of the AgNPs. Moreover, the antibacterial activity against *S. aureus* depends on NP size, where the antibacterial effect increases as the nanoparticle size diameter decrease

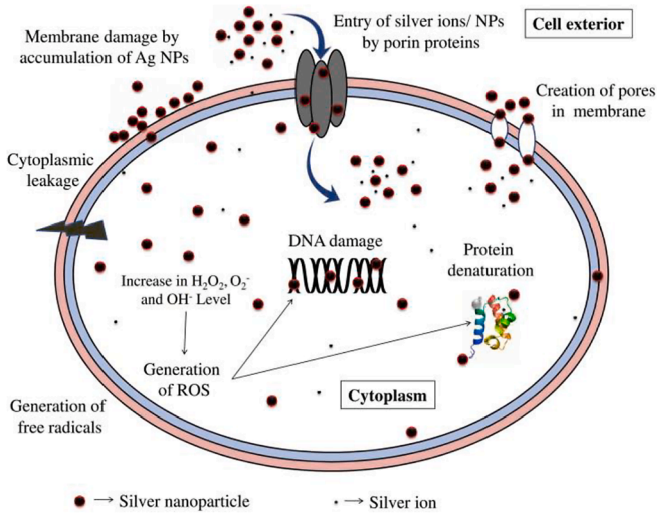

Fig. 9. Schematic representation of the known mechanisms of antibacterial action of silver [139].

[140].

Huang et al. developed an antibacterial silver NP surface-functionalized with d-cysteine. The surface exhibited excellent antibacterial activity against *E.coli, P.aeruginosa* PA01, and *S.aureus*. d-Cysteine inhibited the maturation of biofilm and suppressed bacteria in a dispersed and planktonic unicellular state. Furthermore, d-Cystine increased the lethality of silver NPs to bacteria. This combination of AgNPs and the biofilm-dispersing properties of d-cysteine achieved more durable and more effective antibacterial ability [141]. In contrast to previous research, Sun et al. reported the design and synthesis of perfluoroalkyl attached silica nanoparticles. By adding these NPs into curable coatings, high wear-resistance and antibacterial activity against *E.coli* and *S.aureus* were achieved due to the particle self-migration to the surface [142].

Gadkari et al. developed a silver-loaded CS NP coating by using the layer-by-layer (L-B-L) technique and applied this on cotton fabric. The silver-loaded chitosan (CS-Ag) was prepared by adding an aqueous solution of $AgNO_3$ to a CS nanoparticle suspension. This L-B-L coating exhibited 100% antibacterial activity against both Gram-positive and Gram-negative bacteria. In comparison, the CS-Ag coated fabric showed only 72% and 68% effectiveness against *S. aureus* and *E. coli* [143].

In view of the afore-stated research, the effect of silver NPs can enhance the existing antiseptic properties of materials. Yeray et al. have shown the importance of NP size, where the decrease in nanoparticle size relates to an increase in antibacterial activity [140]. However, it should be noted that smaller particles also exhibit higher cytotoxicity to the host cells. Moreover, Ferdous et al. conclude that biological interaction and toxicity are dependent on particle number and surface area for the same mass of an NP [144]. Huang et al. have shown the importance of combining multiple mechanisms and/or approaches to improve antibacterial activity effectiveness. Further research should focus on minimizing the load of NPs for optimal antibacterial activity in combination with complementary strategies, such as functionalizing silver with d-cysteine. These complementary strategies can increase efficacy, which may lower the concentration of silver NPs needed for optimal antibacterial activity [141].

4. Physical strategies

In this section, the properties of wetted surfaces are first examined, followed by the antibacterial properties of textured surfaces. Exclusive attention is paid to fabricated surfaces that are antibacterial by design free of biocides. The strategies include the fabrication of hydrophilic, hydrophobic, and superhydrophobic topographies.

4.1. Wettability and the contact angle (θ)

Wettability is a key physical parameter used to characterize antibacterial surfaces. The wettability of a surface can be evaluated by measurements of the apparent contact angle (CA^o) of a sessile droplet [145,146]. As shown in Table 3, the CA^o range can aid in identifying the wettability property of the surface. From the CA^o, (super)water repellency [147] or hydrophilicity [148] can be characterized. In most cases, (super)water repellent surfaces can prevent bacterial attachment [149–151]. On the contrary, hydrophilic surfaces might increase bacteria attachment [152] to the surface unless the surface is coated with a slippery material, which in essence retain its hydrophilicity over time [152].

4.1.1. Hydrophobicity and superhydrophobicity

Traditionally, a surface is considered hydrophilic when the CA^o is lower than 90^o, hydrophobic when CA^o is larger than 90^o and smaller than 150^o. Drops with angles lower than 90^o tend to adhere to smooth surfaces. While drops with larger angles than 90^o tend to slide more easily [153]. When the CA^o of a drop is larger than 150^o, the surface is considered superhydrophobic [145,154]. Sessile drops on superhydrophobic surfaces take almost spherical shapes. Superhydrophobicity may not be achieved without micro(nano)topography unless superhydrophobic coatings are used. Chemical techniques enabling superhydrophobic functionality over a surface include chemical etching, templating, sol-gel processing, electro-spinning, anodic oxidation, layer-by-layer assembly, electrochemical deposition, and chemical vapor deposition [48]. Once superhydrophobicity is achieved, a sessile drop might slide off from the surface[154]. Such characteristics can be used to produce self-cleaning materials, providing an additional advantage to reduce bacteria attachment [155,156]. However, understanding wetting properties on antibacterial surfaces is not trivial as complex patterns or other wetting properties can emerge [157,158].

Another point is understanding the action mechanism during self-cleaning because micro(nano)features decorating surfaces are far from smooth. In fact, they are rough, which makes the understanding of the wetting properties challenging and difficult to characterize. The influence of the surface micro(nano)structure on the CA^o of droplets is usually explained by the Wenzel model [159], which applies to wetted surfaces. In other words, the liquid fills in the space between the surface micro(nano)structures. In the Cassie-Baxter model [160], the liquid lies atop the micro(nano)structure, it leaves air between the interspace of the micro(nano)structures under the droplet, and the surface can be considered hydrophobic. A detailed description of the Wenzel and Cassie-Baxter is addressed below.

4.1.1.1. The Young model. The interaction between the solid surface and the liquid drop has been modeled using a number of equations. Assuming that the surface is ideal, i.e., smooth, isotropic and physico-chemically homogenous, the CA^o can be modeled with the Young equation

$$\cos\theta = \left(\gamma_{sg} - \gamma_{sl}\right)/\gamma_{lg}$$

Table 3
Wettability label, range of CA^o, and physical observation. These intervals have been modified to adjust to the CA^o observed in nature.

Wettability	Contact angle^o (θ)	Observation
Hydrophilic	$0^o \leq CA^o \leq 90^o$	The water drop wets the surface. For angles close to zero, the drop spreads on the surface.
Hydrophobic	$90^o < CA^o \leq 150^o$	The water drop partially wets the surface.
Superhydrophobic	$CA^o > 150^o$	The water drop does not wet the surface.

where γ_{sg}, γ_{sl}, γ_{lg} are the energies in the solid-gas, solid-liquid, and liquid-gas interfaces. The Young model does not take into account the influence of surface roughness [161]. Regardless of the topography, the effect of microstructures during wetting Cassie-Baxter and Wenzel models are usually employed to understand droplet-structured surface interaction [147].

4.1.1.2. The Cassie-Baxter model. The Cassie–Baxter model describes the CA° of a drop sitting on top of the microstructures, leaving an air layer under the droplet and around the microstructure. In other words, the Cassie-Baxter can be used to describe a (super)hydrophobic solid; typically, the surface below the drop consists of a fraction of air [147]. Here, it is assumed that the CA° on the purely solid surface can be estimated with the Young equation and can be written as

$$\cos\theta^{*} = \phi_s\,(1 + \cos\theta) - 1$$

where θ* is the apparent CA° corresponding to the state of stable equilibrium, θ is the equilibrium contact on the Young equation, and ϕ_s is the fraction of the liquid interface in contact with the solid.

4.1.1.3. The Wenzel model. The Wenzel model describes the CA° when the liquid has filled the space below the drop. In other words, a sessile drop in a Wenzel state has been impaled on the micro-structures. Unlike the Cassie-Baxter model, the Wenzel model leads to highly adhesive forces in the solid-liquid interface. The Wenzel model can be written as

$$\cos\theta^{*} = r\cos\theta$$

where r is the dimensionless roughness defined as the ratio between the area of the wet surface and the total surface under the droplet [159]. The presented models are the basis for the understanding of surface wetting properties, essential for antibacterial surfaces.

4.2. Antibacterial surfaces in nature

After millions of years of evolution, nature has created unparalleled antibacterial surfaces. Researchers have attempted to mimic hierarchies from nature by employing physical structuring using fabrication methods. Fabrication methods from microelectronics are typically used to mimic the antibacterial effect in nature. Interestingly, the importance of naturally occurring antibacterial surfaces lies in the fact that such are not coated with biocides [41].

4.2.1. Antibacterial leaves

Plants evolved to avoid biofouling on their leaves. Hydrophobicity is their first line to combat the attachment of water drops at the surface. The leaves of plants, such as Taro and Lotus, secrete wax [162–164]. Surfaces covered with bio-wax are hydrophobic, typically displaying contact angles between 74° [164] up to 106° [165]. Such property is thanks to the bio-wax and micro(nano)structure surface features. For example, Taro and Lotus leaves have elliptical bumps whose diameter is around 20 μm [162,163,166]. Such bumps further increase the CA°, making the surface superhydrophobic [163,166,167]. Hierarchical features, whose size is on the order of nanometers, can increase hydrophobicity over leaves [162–164]. By removing the nanocrystals in Lotus leaves, the CA° decreased about 16° [164]. In addition, nano-bio-wax can enhance superhydrophobicity, achieving contact angles up to 142° for the Lotus leaves and up to 150° for the Taro leaves. Furthermore, it has been demonstrated that a higher density of such nanostructures improves the reduction rate of bacteria and bacteria attachment even under water [162,164,168]. Thus, water drops on the surface of leaves slide and fall with relative ease. Self-cleaning arises as an unprecedent strategy because biofouling components may attach to drops rather than staying on the leaves.

4.2.2. Antibacterial skin

Similar to leaves, the skin of some animals evolved to develop anti-biofouling properties, such as micro- and nano-structures and wax. For example, the skin of sharks is covered with micro-structures called riblets. Compared to plants, the typical diameter of riblets is larger than the diameter of the bumps on leaves, between 100 to 300 μm [169,170]. Other topographical surfaces, such as the feet of Gecko, show micro-hairs, called setae, with diameters comprehended between 30 and 130 μm [171]. Other surface topographies like the wing of butterflies display scales similar to shingles, with width between 30 and 50 μm and length between 58 and 146 μm [164]. On a smaller scale, Cicada wings are covered with nanopillars of conical shape [172,173]. It has been found on the wings of cicadas and dragonflies bio-wax [164]. Such bio-wax might play a role in enhancing antibacterials properties along with an increase in hydrophobicity, end-interacting with proteins and lipids components of the cell membrane [174].

To avoid bio-fouling, the surface of leaves developed super-hydrophobicity with contact angles comprehended between 142° and 159° [164]. Meanwhile, the skin of some animals may have considerably lower angles than plants comprehended between 76° up to 147° [164]. Grooming possibly explains such a different range of angles [175], as plants cannot clean their leaves and bio-fouling components remain longer on their surfaces. When a structured surface is super-hydrophilic (CA°=0°), a thin layer of water covers the surface, resulting in a force of attachment to another surface. When a surface is hydrophobic, van der Waals forces produce a tiny force of attachment as well. It has been argued that the numerous structures on the feet of Gecko evolved to optimize the use of both of these forces to attach to walls at will [176].

4.3. Effects of surface modification, micro-, and nano-structures

4.3.1. Fabrication of hydrophobic surfaces

Schwibbert et al. [177] investigated the effect of irregular nano-structures during *E. coli* and *S. aureus* colonization. In this investigation, the nano-structures have been fabricated on polyethylene surfaces using a femtosecond laser. The irregular nano-structures fabricated on the surface led to an increase in the CA° from 65° to 121°. While the topographical surface reduced the adhesion of *E. coli*, the coverage of *S. Aureus* remained unaltered. Schwibbert et al. [177] concluded that bacterial repellence might not only be attributed to wetting properties but the shape and size of the bacteria and the pillars play a role as well. Specifically, *S. aureus* is small enough to fit within the valleys of the surface, unlike *E. Coli*. This allowed the *S. aureus* to adhere to the surface. Nevertheless, the femtosecond laser process has endowed the polyethylene surface with an outstanding antibacterial effect on *E.coli* by impeding bacterial adherence [177].

Peter et al. [32] used direct laser interference patterning (DLIP) to fabricate periodic arrays of cones and holes separated about 850 nm in stain steel. The presence of these structures modified the contact angle of stainless steel from 77±3° to 154±3° for cones and 148±5° for holes. The presence of these structures resulted in a considerable reduction of retention of *E. coli* and *S. aureus*. On *E. Coli*, the retention reduction was about 99.8% for cones and 99.4% for holes. On *S. aureus*, the retention reduction was about 70.6% for cones and 79.1% for holes. However, this nano-fabrication process did not produce superhydrophobic surfaces as drops only slide when the tilting angle reached 90° [32]. Gupta et al. [178] produced stainless steel surfaces with enhanced roughness using a nanosecond pulsed laser. In this investigation, pulses of 100 ns with an energy of 7.5 mJ were used to enhance the contact angle from untreated surfaces from 40° up to 110° after treatment. It was concluded that the laser treatment produced a dual-scale patterning. The first scale is on the micron size, while the second scale is on the nano size. The antibacterial properties of these surfaces were tested with gram-negative *P. aeruginosa* and gram-positive *B. subtilis*. Fluorescent staining on the biofilms of the treated and untreated surfaces confirmed bacterial inactivation and adherence.

Despite not having the self-cleaning attribute of superhydrophobic surfaces, changes in topography on the micro and nanoscale may provide hydrophobic and anti-adhesive properties to stainless steel surfaces. Future research should focus on combining micro-and nano-fabrication with chemical modification techniques to search for synergistic strategies [41,177].

4.3.2. Fabrication of superhydrophobic surfaces

Jalil et al. [31] have produced superhydrophobic surfaces on gold with femtosecond laser pulses. After treatment, the surface exhibited different surface structures on scales of the order of micro and nanometers. Some of the shapes were conic, 1D-rods of less than $\leq$6 μm, and spherical nano-structures with a diameter of $\geq$10 nm. The results indicated that the femtosecond laser-induced changes in the wettability, going from hydrophilic to superhydrophobic. With this technique, treated surfaces demonstrated the reduction of *E. coli* compared to non-treated surfaces [31].

Freschauf et al. [179] developed a shrink method for producing superhydrophobic surfaces using consumer plastics without chemical modification. The method consists of pre-stressed polyolefin (PO) procedure that fabricates rough surfaces with multiscale structures of extreme aspect ratio [180]. PDMS is used to cast such a structure on a mechanically and thermally stable medium. PDMS molds are used to endow various hard plastics with the initial topography. Results indicated that the superhydrophobic surfaces exhibit a significant reduction in bacterial growth of *E. coli* over flat surfaces. The ability to endow superhydrophobic structures to plastics may be a method that is compatible with roll-to-roll manufacturing and scale-up production [179]. Tripathy et al. [181] designed flexible superhydrophobic surfaces decorated with copper hydroxide nanowires. These nanowires are grown separately and transferred onto a polydimethylsiloxane (PDMS) surface by mechanical peeling, allowing non-planar 3D structured surfaces to be fabricated. These surfaces have shown antibacterial effects, blood repellence, hemocompatibility, making them suitable for healthcare applications [181].

Wang et al. [182] fabricated a robust superhydrophobic surface (Fig. 10) for possible long-term applications in the health care setting. Here, both of these apparently opposite characteristics are achieved by fabricating a micro-structured armor around the nano-structures. While the nano-structures provide superhydrophobicity, the micro-structured armor provides durability. The micro-structured armor is an interconnected frame containing 'pockets' with water-repellent and mechanically fragile nanostructures. When such composite structures are fabricated on the surface of various materials such as silicon, ceramic, metal, and transparent glass, super-hydrophobicity has persevered after abrasion. Silicon inverted-pyramidal microstructures were manufactured by photolithography. However, ceramic, metal, and transparent glass inverted-pyramidal microstructures were manufactured by embossing technology. Interestingly, the produced structures showed high mechanical stability as superhydrophobic interaction was maintained after extreme harsh conditions [182].

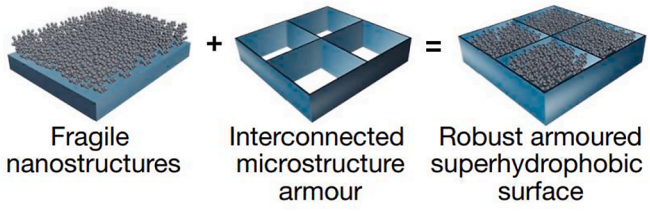

Fig. 10. Strategy for enhancing the mechanical stability of the superhydrophobic surface by housing water-repellent nanostructures within a protective microstructure 'armor' [182].

4.4. Physical grounds behind the bactericidal effect

The biocidal effects of topographical surfaces have been investigated deeply. Some of these studies have focused on natural surfaces, while others mimic surfaces from nature. Recently, the skin of sharks inspired the fabrication of antibacterial surfaces [183–185]. These topographical surfaces consist of millions of uniquely ordered micropatterns that provide antibacterial and antifouling properties [184]. In some cases creating bacterial patterns over the structured surface [157]. It has been observed that micropatterns similar to the sharkskin strongly inhibit the biofilm formation of *P. aeruginosa* and *S. aureus* [183] without using toxic compounds [184]. The microtopography of shark skin not only inhibits the attachment of bacteria in an early stage but also inhibits biofilm formation [186]. Bacterial loads are reduced due to the effect of longitudinal vortices formed on the grooves of the riblets [185]. Such vortices are the result of a turbulent flow induced by non-uniform grooves. In reverse osmosis membranes, the optimal length of sharklets and the space between them has been optimized at 2 μm [187].

Moving on from shark-like riblets, cicada wings containing nanostructures have also been proposed as a powerful alternative applied in the field of antibacterial surfaces [188,189]. The conical nanopillars on cicada wings are protruding upward from the surface. Biophysical models of the bactericidal activity reported that the cell wall attaches to these conical pillars during drying. As the drying process continues, the cell wall is ruptured due to extension stress [189,190]. The stress concentrates in the regions suspended between the pillars, ultimately tearing the membrane. These natural surfaces more effectively kill gram-negative bacteria than gram-positive bacteria. It is believed that gram-positive bacteria are more resistant due to the higher stiffness of their cell wall [164,191]. Another investigation of *E. coli* on the wings of dragonflies demonstrated that the membrane is damaged even before direct contact with the structures takes place [192]. This investigation demonstrated that membrane damage results from a combination of strong adhesion between the nanopillars and the extracellular polymeric substance. An additional shear force appears when immobilized bacteria attempt to colonize these topographies.

5. Multifunctional antibacterial strategies: coatings and topographies

Multiple functions can enhance antibacterial properties at the surface to combat biofilm formation and prevent bacterial infections. Cloutier et al. have categorized multifunctional antibacterial surface strategies into multiapproach, multiagent release, and multi-property surfaces [193]. In this review, we focus on multiapproach and multi-property surfaces. An additional level of multifunctionality is added with the integration of physical structuring, addressed in the perspective section.

5.1. Multi-approach surface

This is a novel strategy with high potential in antibacterial coatings. The approach uses multiple functional routes, such as CA, BAR, and AA, to improve antibacterial efficacy. Various efforts have been made to develop multi-approach surfaces over the last few decades [194]. One of the main disadvantages of BAR, AA, and TM is that dead bacteria can build up and start biofilm formation [10]. Townsend et al. have developed a dual approach coating consisting of two types of AMPs for implants. This dual system consists of electrostatic released peptides that can sterilize surrounding tissue in the short term and works in tandem with covalently attached peptides for long-term bacterial inhibition [195]. He et al. demonstrated an outstanding novel antibacterial and antifouling strategy whereby a surface structure, consisting of a CA upper-layer and an AA sub-layer, is made. This surface is prepared by casting gemini quaternary ammonium salt (GQAS) waterborne polyurethanes over layered PEG and hydrophobic blends. The authors

speculate that GQAS brushes are deployed at the polymer-air interface forming an antibacterial upper-layer. The combination of GQAS and the antifouling sub-layers, such as PEG, endow long-lasting antifouling properties and maintain bacterial killing efficiency close to 99%. This is a dual approach of AA and CA principles that can promote surface roughness [196]. However, the roughness obtained by GQAS/PEG/hydrophobic surface does not compare to physical structuring methods, such as laser patterning [31] or microfabrication [197], which have more control over the micro/nanoscale. Advancements in the field can be made by combining AA and CA with physical structuring methods. For instance, structuring could enhance water repellency, enabling higher biofouling, maintaining AA and CA bacteria-killing properties active.

5.1.1. Switchable surfaces

Antibacterial switchable surfaces are based on the ability to switch functions, and therefore can be considered within multi-approach. Such a level of multifunctionality can, for example, promote bacteria-killing and bacteria-releasing [198,199]. Bacterial-killing and bacteria-release properties can be activated by either temperature or light [200,201]. The application of an electrical field or other substances such as salt and sugar or variations in pH are important stimuli that enable switching between antibacterial functions [202–204]. In Table 4, a summary of the most representative switching functionalities found in the literature is provided. This table shows the great variety of switching approaches that can modulate bacterial viability at the surface.

A few examples are described in the following lines to highlight the bacteria-killing and bacteria-release properties modulated using temperature or pH. For other stimuli than temperature and pH, the reader should consult the references listed in Table 4. G. Cheng et al., demonstrated that a change in pH could trigger switchable properties. In this case, the coating located at the surface has bacteria-killing and antifouling properties. The antibacterial agent was a cationic pCBMA-1 C2. Then, by changing the pH, the cationic deviated is hydrolyzed to a zwitterionic, releasing the bacteria from the surface [206]. Q. Yu et al. have developed a nanopatterned thermoresponsive surface (Fig. 11). The nanopattern is composed of a thermoresponsive polymer, poly(N-isopropylacrylamide) (PNIPAAm), and a QAC that act as a biocide [216]. The patterned QAC does not only modulate the killing of the bacteria using QAC as a tethered biocide, but PNIPAAm aids to release the bacteria from the nanotopography. Therefore, PNIPAAm can be classified as a fouling-release polymer [228,229]. Besides the functionality given by the PNIPAAm and QAC, the effect of topography (Fig. 11) might also play an important role during bacteria-killing [230] or release [216].

Table 4
Switching functionalities that promote bacteria-killing and bacteria-release [205][a].

Bacterial killing	Bacterial Release	Mechanism of bacterial release	Reference
Cationic CB esters	CB esters	pH	[206,207,208]
SA			[209]
Cationic Arg-Est	Arg-Est		[210]
Cecropin B	PMAA		[211]
Lysozyme			[212]
Cationic precursors	ONB esters	UV light	[213,214]
Cationic PSBEDOT-Ox	PSBEDOT	Electrical potential	[215]
QAC	PNIPAAm	Temperature	[216,217]
Lysozyme			[218,219]
AgNPs			[220,221]
Cationic PTMAEMA	PTMAEMA	Counterion	[222]
TCS or PolyTA	PDVBAPS	Salt	[223,224]
CD-QAC	PBA/CD-QAC	Fructose	[225]
TRGO	Ada/ManCD	AdCNa	[226]
GNPL	PTLF	Vc	[227]

[a] Abbreviations: Ada, adamantine; AdCNa, sodium adamantine carboxylate; Arg-Est, L-arginine methyl ester–methacryloylamide; CB, carboxybetaine; CD, β-cyclodextrin; GNPL, gold nanoparticle layer; ManCD, heptamannosylated CD; ONB, *o*-nitrobenzyl; PBA, phenylboronic acid; PDVBAPS, poly(3-(dimethyl(4-vinylbenzyl)ammonium)propyl sulfonate); PMAA, poly(methacrylic acid); PNIPAAm, poly(*N*-isopropylacrylamide); PSBEDOT, poly(sulfobetaine-3,4-ethylenedioxythiophene); PSBEDOT-Ox, oxidized PSBEDOT; PTLF, phase-transitioned lysozyme film; polyTA, poly[2-(tert-butylamino) ethyl methacrylate]; PTMAEMA, poly((trimethylamino)ethyl methacrylate chloride); QAC, quaternary ammonium salt; SA, salicylate acid; TCS, triclosan; TRGO, thermally reduced graphene oxide; Vc, vitamin C.

5.2. Multi-property surfaces

Multi-property surfaces can display several properties simultaneously, such as self-healing, anti-frost, anti-fog properties, or increased mechanical strength. Wei et al. developed a universal strategy by alternating L-B-L deposition of a polyanion, poly(acrylic acid-co-1-adamantan-1-ylmethyl acrylate) [P(AAco- adamantane)] with guest adamantane groups, and a polycation, poly-(allylamine hydrochloride) (PAH). The guest adamantane groups served as anchor points for the immobilization of functional host molecules by supramolecular forming host-guest complexes with β-cyclodextrin (β-CD) and derivatives modified with quaternary ammonium salt groups (CD-QAS). In conclusion, due to the nature of non-covalent host-guest interaction, versatility in incorporating bio-molecules without impairing functionality was achieved. In addition, dead bacteria are easily removed, and CD-QAS and its derivates can be regenerated [231]. Guo et al. developed a multifunctional antibacterial self-cleaning surface coating. This novel coating is developed by grafting poly(N-vinylpyrrolidone-co-maleic anhydride) (poly(NVP-co-MA)) co-polymer on glass slides. This multifunctionality is achieved due to PMA segments attaching covalently to the glass surface to enhance long-term stability, whereas PVP segments enable anti-fog, anti-frost, antibacterial, and self-cleaning properties. The coating exhibited antibacterial activity against *E.coli* and *S.aureus,* robustness, durability, and could shield the surface from fog, frost, and oil-based contaminants [232].

6. Perspective: towards long-term antibacterial activity

At first, future research should address the standardization of methods used to address challenges such as long-term antibacterial activity and stability, reduced toxicity, increased biocompatibility, and wettability. Another issue is the gap of knowledge on surface-attached QACs AMPs and QSI. Mentioning QAC specifically, its use is constant in surfaces because of the antibacterial effectiveness it presents for long periods [185]. AMPs studies only mention effectiveness after 24 hours of exposed bacteria, and more studies are needed to check how various antimicrobial surfaces perform over a longer time [186]. As for nanoparticles, the antibacterial effectiveness has been assessed until day 7 [187,188]. Although these strategies show high potential for their long-term stability and high efficacy, longer times are required.

It is also important to understand that, while studies have combined the principles of AA, CA, and BAR in recent years, few have investigated

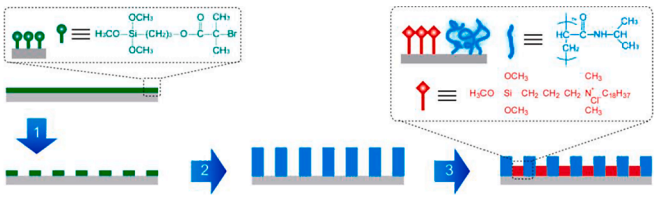

Fig. 11. Depiction of the procedure for the preparation of nanopatterned PNIPAAm surfaces (Steps 1 and 2) and nanopatterned PNIPAAm/QAC surfaces (Steps 1–3) [216].

these principles in conjunction with TM. Those above chemical and physical modification techniques have great potential in creating long-term multifunctional antibacterial surfaces. One of the major issues might be that the topography substructure (e.g., nanotopogaphy) becomes coated, losing its antibacterial properties. However, if the coating is effectively applied to the topography without altering the antibacterial efficacy, it could grant outstanding biocompatibility, enhanced antibacterial functionality, antifouling, or even self-healing properties. The integration of TM in conjunction with chemical modification techniques will require advanced knowledge but, in return, can become a new standard of an application controlling biofouling in several fields of application such as bio-medical, food industry, diagnostics, and marine-related industry.

7. Conclusion

Despite the increasing knowledge on antibacterial strategies, many approaches fall short in providing long-term stability. Understanding surface-attachment mechanisms or how dead, attached bacteria to the surface could be removed via self-cleaning has significantly progressed in the development of new anti-biofilm strategies. Many of the presented reports show exceptional antibacterial or anti-adhesion properties. However, only a few have shown their potential for long-term application. Future perspectives and challenges can be addressed when combining multiscale functionalities to combat bacterial attachment and biofilm formation. Future studies might consider not assessing a variety of bacterial strains, including drug-resistant strains, to show an advantage when compared with available antimicrobial strategies.

Declaration of Competing Interest

No conflict of interest exists.

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